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Journal
Movement Disorders, 27(13)

ISSN
0885-3185

Authors
Goldman, Samuel M
Kamel, Freya
Ross, G Webster
et al.

Publication Date
2012-11-01

DOI
10.1002/mds.25216

Peer reviewed

Published in final edited form as:
Mov Disord. 2012 November ; 27(13): 1652–1658. doi:10.1002/mds.25216.

Genetic Modification of the Association of Paraquat and Parkinson's Disease

Samuel M. Goldman, MD, MPH[1,*], **Freya Kamel, PhD, MPH**[2], **G. Webster Ross, MD**[3], **Grace S. Bhudhikanok, PhD**[1], **Jane A. Hoppin, ScD**[2], **Monica Korell, MPH**[1], **Connie Marras, MD, PhD**[4], **Cheryl Meng, MS**[1], **David M. Umbach, PhD**[5], **Meike Kasten, MD**[6], **Anabel R. Chade, MD**[7], **Kathleen Comyns, MPH**[1], **Marie B. Richards, PhD**[8], **Dale P. Sandler, PhD**[2], **Aaron Blair, PhD**[9], **J. William Langston, MD**[1], and **Caroline M. Tanner, MD, PhD**[1]

[1]The Parkinson's Institute, Sunnyvale, California, USA

[2]Epidemiology Branch, National Institute of Environmental Health Sciences, National Institutes of Health (NIH), Department of Health and Human Services (DHHS), Research Triangle Park, North Carolina, USA

[3]Veterans Affairs Pacific Islands Health Care System, Honolulu, Hawaii, USA

[4]Toronto Western Hospital, University of Toronto, Toronto, Ontario, Canada

[5]Biostatistics Branch, National Institute of Environmental Health Sciences, NIH, DHHS, Research Triangle Park, North Carolina, USA

[6]Departments of Neurology and Clinical and Molecular Neurogenetics, University of Lü beck, Lü beck, Germany

[7]Institute of Cognitive Neurology, Institute of Neuroscience, Favaloro University, Buenos Aires, Argentina

[8]Westat Inc., Durham, North Carolina, USA

[9]Occupational and Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, DHHS, Bethesda, Maryland, USA

Abstract

Paraquat is one of the most widely used herbicides worldwide. It produces a Parkinson's disease (PD) model in rodents through redox cycling and oxidative stress (OS) and is associated with PD risk in humans. Glutathione transferases provide cellular protection against OS and could potentially modulate paraquat toxicity. We investigated PD risk associated with paraquat use in individuals with homozygous deletions of the genes encoding glutathione *S*-transferase M1 (GSTM1) or T1 (GSTT1). Eighty-seven PD subjects and 343 matched controls were recruited from the Agricultural Health Study, a study of licensed pesticide applicators and spouses in Iowa and North Carolina. PD was confirmed by in-person examination. Paraquat use and covariates were determined by interview. We genotyped subjects for homozygous deletions of *GSTM1* (*GSTM1*0*) and *GSTT1* (*GSTT1*0*) and tested interaction between paraquat use and genotype using logistic regression. Two hundred and twenty-three (52%) subjects had *GSTM1*0*, 95 (22%) had *GSTT1*0*, and 73 (17%; all men) used paraquat. After adjustment for potential confounders,

[*]Correspondence to: Samuel M. Goldman, The Parkinson's Institute, 675 Almanor Avenue, Sunnyvale, CA 94085, USA; sgoldman@thepi.org.

Relevant conflicts of interest/financial disclosures: Nothing to report.

Full financial disclosures and author roles may be found in the online version of this article.

there was no interaction with *GSTM1*. In contrast, *GSTT1* genotype significantly modified the association between paraquat and PD. In men with functional *GSTT1*, the odds ratio (OR) for association of PD with paraquat use was 1.5 (95% confidence interval [CI]: 0.6–3.6); in men with *GSTT1*0*, the OR was 11.1 (95% CI: 3.0–44.6; *P* interaction: 0.027). Although replication is needed, our results suggest that PD risk from paraquat exposure might be particularly high in individuals lacking GSTT1. *GSTT1*0* is common and could potentially identify a large subpopulation at high risk of PD from oxidative stressors such as paraquat.

Keywords

Parkinson's disease; paraquat; glutathione transferase; pesticide; gene-environment interaction

Oxidative stress (OS) has long been implicated as a key pathophysiologic mechanism in the etiology of Parkinson's disease (PD).[1,2] Individuals with sporadic PD manifest increased levels of reactive oxygen species (ROS) and reduced antioxidant capacity,[3] and the rare monogenic forms of PD associated with mutations in the *alpha-synuclein, PARKIN, PINK1*, or *DJ-1* genes may also involve OS.[4,5]

The cause of most PD is likely to be multifactorial, with both genes and environment contributing to disease risk.[6] Pesticide use is among the most consistently associated environmental risk factors for PD, but only a few specific compounds have been implicated.[7,8] We recently reported on a significantly increased risk of PD associated with use of the common herbicide, paraquat, in a case-control study nested in a large cohort of licensed pesticide applicators and their spouses.[9] A structural analog of the dopaminergic neurotoxin, MPP^+, paraquat induces OS through redox cycling and produces a selective animal model of parkinsonism that recapitulates major pathological features of PD.[10–12]

Glutathione *S*-transferase M1 (GSTM1) and T1 (GSTT1) are highly conserved members of a class of cytosolic enzymes that detoxify a wide range of xenobiotic compounds by catalyzing the conjugation of glutathione to electrophilic substrates.[13,14] GSTs also metabolize endogenous compounds, such as lipid hydroperoxides and catecholamine oxidation by-products, that form during OS, and they prevent redox cycling. [14–16] GSTs are expressed in a broad range of human tissues, including liver, gut, and brain, and are upregulated in response to paraquat exposure.[13,17–22] Approximately 50% of Caucasians lack functional GSTM1, and 20% lack functional GSTT1 as a result of homozygous deletions of the *GSTM1* (designated *GSTM1*0* or "*GSTM1* null") and *GSTT1* (*GSTT1*0*) genes, respectively. Frequencies of homozygous deletions in other ethnic groups may be even higher.[23]

We hypothesized that deficient function of metabolic enzymes involved in the response to OS might enhance the neurotoxicity of paraquat exposure and thus the risk for developing PD. The present study tested the hypothesis that the association between paraquat and PD risk is enhanced in those with homozygous deletions of *GSTT1* or *GSTM1*.

Subjects and Methods

Subject Ascertainment

The Farming and Movement Evaluation study (FAME) is a case-control study nested in the Agricultural Health Study (AHS).[9,24] The AHS is a prospective study of licensed pesticide applicators (mostly farmers) and their spouses recruited in 1993 to 1997 in Iowa and North Carolina (n = 84,739).[25] FAME participants were identified from AHS data releases P1REL0506 and AHSREL06 (http://aghealth.nci.nih.-gov/).

Cases—AHS cohort members suspected to have PD were identified by screening questionnaire or state mortality records. As part of FAME, neurologists assessed suspect case subjects at home. Assessments included a standardized neurological history, examination, and scripted videotaping. PD diagnosis was determined by consensus of two movement disorder specialists using all available information, including medical records, and applying National Institute of Neurological Disorders and Stroke/UK Brain Bank criteria.[26,27]

Controls—Potential control subjects were identified by stratified random sampling of all living, nondemented AHS participants not suspected to have PD and were frequency-matched to case subjects by age, gender, and state (Iowa or North Carolina) at a ratio of approximately 3 per case. Neurologists or technicians trained by neurologists conducted control assessments, which included scripted videotaping of their movements. Technician-assessed controls with possible parkinsonism were reassessed by neurologists. Eighty-eight percent of "suspected" cases and 71% of eligible controls participated, and a total of 115 PD cases and 383 control subjects were enrolled.[9] DNA was unavailable for 28 subjects (15 cases [13%] and 13 controls [3.4%]), and genotyping was unsuccessful in 1 control. In those successfully genotyped, paraquat usage could not be determined for 10 cases (10%) and 24 controls (6.5%), and an additional 3 cases and 2 controls lacked smoking data. The present analysis includes 87 cases and 343 controls with complete data.

FAME was approved by institutional review boards for the Parkinson's Institute, National Institutes of Health, and its contractors. All participants provided written informed consent.

Data Collection

Exposure Assessments—Trained interviewers at the Parkinson's Institute used structured computer-assisted telephone interviews (CATIs) to collect demographic and detailed lifestyle information, including cigarette smoking and history of head injury. We also collected complete lifetime occupational histories that included all farm jobs held after age 14 as well as detailed information on pesticide use in those jobs. We used proxy informants for subjects who were unable to complete interviews because of death (after blood collection), hearing or speech deficits, or cognitive impairment.[28] Exposures were assessed until a reference age, defined as age at diagnosis for cases, and as median case diagnosis age in the corresponding gender-, state-, and age-specific stratum for controls. Using information from the CATI interviews, we determined whether subjects ever used paraquat (mixed or applied one or more times) and cumulative lifetime years of use. Self-reported paraquat use before 1962, when paraquat was first marketed in the United States, was excluded. Cigarette smoking was defined as smoking at least one cigarette daily for 6 months or longer. Head injury was defined as an affirmative response to the question, "Have you ever had a head injury where you lost consciousness or were diagnosed with a concussion by a doctor?"

Genotyping—DNA was extracted from venous blood collected during the in-home exams.[29] Genotyping was conducted by the genomics core at the University of California San Francisco (San Francisco, CA). We tested for homozygous deletions of *GSTT1* and *GSTM1* using fragment-length multiplex polymerase chain reaction.[30] The assay did not distinguish heterozygotes from non-null homozygotes, and these are classified as *GSTT1*1* and *GSTM1*1*, respectively.

Statistical Analyses

We compared participant characteristics using Fisher's exact test or Pearson's chi-square statistic for categorical data and independent *t* tests or Mann-Whitney's/Wilcoxon's rank-

sum tests for continuous data. All reported *P* values are two-tailed. Associations between PD and paraquat or *GSTM1* and *GSTT1* genotypes were tested using unconditional logistic regression. To control for potential confounding, we included reference age (tertiles), gender, state (IA or NC), and cigarette smoking (ever/never) in all models. We tested multiplicative interaction between ever using paraquat and *GSTM1*0* or *GSTT1*0* by including a product term in logistic models and calculated odds ratios (ORs), 95% confidence intervals (CIs), and *P* values using exact methods. No women used paraquat; therefore, we conducted analyses of interaction only in men. We also considered three classes of cumulative lifetime years of use, defined as never used, used less than or equal to median number of years (≤4 years), or used greater than median number of years based on distribution in controls. Trend across classes was assessed by including a continuous variable (with values 1, 2, and 3 for each class, respectively) in logistic models. In sensitivity analyses, we examined whether adjusting for educational level, respondent type (subject or proxy), head injury, or race/ethnicity changed inferences. We also adjusted for overall pesticide use by including a variable for use of any pesticide for more than 25 lifetime days, and we conducted analyses restricted to non-Hispanic whites or that excluded subjects with a history of PD in a first-degree relative. In addition, sensitivity models included terms for *GSTM1*0* and *GSTT1*0* simultaneously and tested for interaction between *GSTM1*0* and *GSTT1*0*. We tested the fit of models with and without sensitivity variables using likelihood-ratio tests. In cases, we compared age at PD diagnosis within strata defined by paraquat exposure and GST genotype using linear regression. Statistical analyses were conducted with *SAS* (version 9.1.3; SAS Institute, Cary, NC) and *SPSS* software (version 12.0; SPSS, Inc., Chicago, IL).

Results

Eighty-seven case and 343 control subjects had both genotype and exposure data (Table 1). Demographic characteristics of subjects with complete and incomplete data were similar, and the frequency of missing paraquat usage data was similar in those with null and non-null genotypes (data not shown). Case and control subjects were well matched on age, duration from index date until interview, gender, state, and ethnicity. Ninety-eight percent of subjects were non-Hispanic white. Case subjects required a proxy informant more frequently than controls (17% versus 1%), and a larger proportion reported a first-degree relative with PD (14% versus 7%). Twenty-one percent of controls had *GSTT1*0* genotype and 53% had *GSTM1*0*, consistent with their expected population frequencies.[23]

PD risk was modestly increased in those with *GSTT1*0* genotype, but the association was not significant (Table 2). Conversely, *GSTM1*0* was associated with a *reduced* PD risk. Seventy-three subjects (21 cases and 52 controls), all men, reported ever mixing or applying paraquat. Proxy respondents for case and control subjects endorsed paraquat use with similar frequency (4 of 15 and 1 of 3, respectively). As previously reported,[9] PD risk was significantly associated with ever use of paraquat, and risk increased with cumulative years of use (*P* trend: 0.004).

We found significant multiplicative interaction between use of paraquat and *GSTT1* genotype (Table 3). The risk of PD in men with *GSTT1*1* who used paraquat was only modestly elevated (OR, 1.5; 95% CI: 0.6–3.6), whereas risk was markedly elevated in men with *GSTT1*0* (OR, 11.1; 95% CI: 3.0–44.6; *P* interaction: 0.027). Relative excess risk from interaction (RERI) in an additive model was similarly elevated (RERI, 9.5).[31] Risk associated with *GSTT1*0* and paraquat use was at least as great in analyses restricted to non-Hispanic white men (OR, 11.5; 95% CI: 3.1–46.9; *P* interaction: 0.021) or men without a family history of PD (OR, 13.4; 95% CI: 3.3–62.6; *P* interaction: 0.022). Inclusion of *GSTM1* genotype in regression models had minimal effect on the interaction between

paraquat use and *GSTT1* genotype (data not shown). Greater total years of paraquat use was strongly associated with increasing risk of PD in those with *GSTT1*0* (*P* trend: 0.001), but not in those with *GSTT1*1* (data not shown).

Results were similar in analyses adjusted for respondent type (subject or proxy), ethnicity, head injury, education, greater than minimal usage of any pesticide, or when restricted to subjects with PD duration of 7 years or less (data not shown).

We found no evidence of statistical interaction between paraquat and *GSTM1*; PD risk was similar to the expected risk if paraquat and *GSTM1* genotype were acting as independent risk factors in either additive or multiplicative models. Inclusion of *GSTT1* or other variables had little effect on this relationship.

Among PD cases, age at diagnosis did not differ by *GSTT1* or *GSTM1* genotype (data not shown). Men who used paraquat were non significantly younger at diagnosis than those who did not (58.7 versus 62.3 years, respectively; $P = 0.1$), but among paraquat users, age at PD diagnosis did not differ by GST genotype.

Discussion

The association of paraquat use with PD risk was highly dependent on *GSTT1* genotype. Risk associated with paraquat use was 7.4-fold greater in men with *GSTT1*0* than in those with *GSTT1*1*, and we observed a significant dose-response relationship in *GSTT1*0* carriers. In contrast, we did not observe interaction between paraquat use and *GSTM1* genotype.

GSTT1 and *GSTM1* gene deletions are very common. Approximately 20% of Caucasian and 50% of Asian populations have no detectable GSTT1 enzyme, whereas 50% of both populations lack GSTM1.[23] Associations of *GSTT1*0* and *GSTM1*0* with PD risk have been very inconsistent, possibly reflecting environmental heterogeneity.[32–40] In the present study, when paraquat exposure was not considered, *GSTT1* and *GSTM1* genotypes were only marginally associated with PD risk. Somewhat surprisingly, *GSTM1* deletion was inversely associated with PD risk. Although a cautionary finding, others have reported similar results,[32,41] consistent with observations that GSTM1 can sometimes bioactivate xenobiotics, increasing their toxicity[42]—as reported in studies of pesticide exposure and renal carcinoma.[43]

To the best of our knowledge, no previous epidemiologic studies of PD have investigated paraquat interaction with metabolic genetic variants. Two studies assessed interaction of *GSTT1* with exposure to pesticides in general.[35,41] Although neither reported significant interaction, similar to our results, Dick et al. found that PD risk associated with pesticide exposure was higher in those with *GSTT1*0*, but lower in those with *GSTM1*0*.

Previous studies have reported that several other metabolic genes may also modify pesticide associations with PD, including *CYP2D6* (cytochrome P450 2D6),[44] *GSTP1* (glutathione-S-transferase P1),[45,46] *NQO1* (nicotinamide adenine dinucleotide phosphate dehydrogenase),[47] *SOD2* (manganese superoxide dismutase), [47] and *PON1* (paraoxonase 1).[48] However, none of these studies specifically assessed interactions with paraquat.

Paraquat has been commercially available since 1962[49] and is one of the most widely used herbicides worldwide.[50] Exposure has been associated with PD in most,[9,24,51–53] but not all,[54,55] previous studies. A structural analog of the dopaminergic neurotoxin, MPP^+, paraquat generates ROS through redox cycling. [56,57] Consistent with etiopathologic hypotheses of PD, paraquat decreases levels of reduced glutathione (GSH) in the SN and

striatum, increases lipid peroxidation, and damages mitochondria in the central nervous system and systemically.[58–62] In rodent models, repeated administration of paraquat produces pathologic changes associated with PD, including alpha-synuclein (α-Syn) aggregation and selective nigral injury.[10,12,63,64]

Although paraquat is thought to be poorly metabolized and is probably not a direct substrate of GST,[49] depletion of GSH enhances paraquat toxicity,[65] whereas administration of GSH may attenuate it.[66] In addition, repeated exposure to paraquat or other pesticides or oxidative stressors induces expression of GSTT1,[21,22,67–69] suggesting that upregulation of *GSTT1* in response to an oxidative challenge may impart protection against ROS more generally. Moreover, in *Drosophila* models of PD, GST loss-of-function alleles enhance dopaminergic neuron loss and motor dysfunction in α-Syn overexpressors or *parkin* mutants.[70,71]

In addition to conjugating electrophilic xenobiotic substrates, GSTs play a role in biosynthesis of leukotrienes, prostaglandins, and steroid hormones and interact with signaling molecules affecting gene expression, including the peroxisome proliferator-activated receptor gamma, nuclear factor-erythroid 2 p-45-related factor 2, and nuclear factor kappa-beta. GSTT1 also shares homology with several stress-related proteins, including p28, which is involved in cellular redox homeostasis.[72,73]

Our study has some limitations. First, the number of individuals with *GSTT1*0* genotype who used paraquat was small (n = 15), and risk estimates for joint effects are imprecise. Nonetheless, despite the possibility of a false-positive finding, lower confidence limits are >1, and results are compatible with at least a 3-fold increase in risk. Second, because most participants were exposed to a number of other pesticides, we cannot exclude effects of agents other than paraquat or rule out the possibility that our results are from combined exposures. However, previous analyses of FAME data that evaluated multiple pesticides found significant links only with use of paraquat or rotenone, [9] and in the present study, the interaction between *GSTT1* and paraquat remained after adjustment for either rotenone or overall pesticide use. Third, paraquat use was determined by self-report and could be subject to misclassification, but exposure classification was based on complete lifetime occupational histories, rather than response to a single question, and AHS licensed pesticide applicators provide reliable recall of specific agents they have previously used.[74] In addition, exposure misclassification would most likely diminish ORs, rather than increase them.[75] Furthermore, exposure misclassification is not likely to vary by *GSTT1* genotype and therefore would not explain the observed interaction. Fourth, we included prevalent PD cases still living at AHS enrollment, so survivor bias is possible. However, results were similar when restricted to subjects with shorter disease duration. Finally, reliance on proxy informants for a larger proportion of case subjects than control subjects could have introduced bias, but similar proportions of proxy respondents for case and control subjects reported paraquat use, and associations persisted in regression models adjusted for respondent type.

Strengths include the use of an agricultural cohort with a relatively large number of paraquat-exposed subjects, the quality of diagnosis, which was based on in-person assessment and agreement of movement disorders experts, and the completeness and reliability of the pesticide exposure information. An additional strength is the nested case-control design with an internal control group who had similar exposure opportunities as the cases and similar demographic and lifestyle characteristics, reducing the likelihood of confounding.

Although the number of exposed study subjects was small and results should be considered preliminary, our findings suggest PD risk from paraquat exposure may be extremely high in concert with GSTT1 deficiency. *GSTT1* gene deletions are very common, and if our results

are replicated, carriers could potentially represent a large population at high risk of PD from environmental toxicants, such as paraquat.[23] In addition to replication of our findings in other well-characterized study populations, future work should investigate potential gene-dosage effects in subjects with heterozygous deletions of *GSTT1* as well as interaction with other enzymes involved in the metabolism of paraquat and defense against OS.

Acknowledgments

The authors thank Ms. Debbie McCullough for her statistical support and the participants of the FAME study.

Funding agencies: This research was supported, in part, by the Intramural Research Program of the National Institutes of Health, National Institute of Environmental Health Sciences (NIEHS) grants (Z01-ES044007 and Z01-ES049030), a National Cancer Institute grant (Z01-CP010119), NIEHS grants R01-ES10803 and U54 ES012077, the Michael J. Fox Foundation, and James and Sharron Clark.

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TABLE 1

Subject characteristics

Characteristics	Cases (n = 87)	Controls (n = 343)
Reference age,[a] mean (SD), range	61.4 (9.1), 45–87	61.5 (7.6), 45–80
FAME enrollment age, mean (SD), range	68.7 (8.4), 48–89	69.1 (8.2), 42–88
Years from reference date until exam, mean (SD), range	7.6 (5.2), 0–22	7.8 (4.5), 0–22
State, n from Iowa (%)	67 (77)	247 (72)
Male, n (%)	63 (72)	261 (76)
Non-white or Hispanic, n (%)	2 (2.3)	8 (2.3)
n (%) missing race/ethnicity data	0	6 (1.7)
Proxy respondent, n (%)	15 (17)	3 (1)
PD in first-degree relative, n (%)	12 (14)	22 (7)
Education, mean years (SD)	12.6 (2.1)	12.6 (2.1)
Cigarette smoker, n (%)	19 (22)	123 (36)
Head injury, n (%)	20 (24)	60 (18)
*GSTT1*0*, n (%)	24 (28)	71 (21)
*GSTM1*0*, n (%)	41 (47)	182 (53)
*GSTT1*0* and *GSTM1*0*, n (%)	9 (10)	26 (8)
Paraquat use, n (%)	21 (24)	52 (15)

[a]Age at diagnosis for cases; median case diagnosis age in the corresponding gender-, state-, and age-specific stratum for controls.

Abbreviation: SD, standard deviation.

TABLE 2

Risk-factor-associated ORs for PD (95% CI)

Variable	All Subjects[a] (Case n = 87; Control n = 343)	Men[b] (Case n = 63; Control n = 261)
Cigarette smoking	0.5 (0.3–0.9)	0.4 (0.2–0.8)
*GSTT1*0* genotype	1.5 (0.9–2.6)	1.7 (0.9–3.2)
*GSTM1*0* genotype	0.8 (0.5–1.3)	0.5 (0.3–0.9)
Paraquat use (ever versus never)	—[d]	2.6 (1.3–5.0)
Paraquat total years of lifetime use:		
Never used	—[d]	1.0 (ref)
Used ≤ median[c]		2.5 (1.1–5.8)
Used > median		3.1 (1.3–7.2)
P trend		0.004

[a] Adjusted for state, age, smoking, and gender.

[b] Adjusted for state, age, and smoking.

[c] Median use = 4 years.

[d] No women used paraquat.

TABLE 3

Interaction between *GSTT1* genotype and paraquat use in men[a]

Paraquat Use	*GSTT1* Genotype	Case n	Control n	OR[b] (95% CI)
No	*GSTT1*1*	32	160	Ref
Yes	*GSTT1*1*	12	46	1.5 (0.6–3.6)
No	*GSTT1*0*	10	49	1.1 (0.4–2.4)
Yes	*GSTT1*0*	9	6	11.1 (3.0–44.6)
P interaction				0.027

[a] Exact logistic regression.

[b] Adjusted for state, age, and smoking.